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**Coastal Benthic Optical Properties (CoBOP): Optical Properties
of Benthic Marine Organisms and Substrates**

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20 New England Business Center,
Andover, MA 01810

2002

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SUMMARY

Research was conducted as part of the ONR Departmental Research Initiative in Coastal Benthic Optical Properties (CoBOP). The long-term objective of our research is to gain an understanding of the nature and significance of fluorescence and reflectance characteristics of benthic marine organisms in general, and coral reef cnidarians in particular. We wish to determine how biological processes act to produce the optical properties and how optical measurements can be used to provide insight into biological state or process. The specific goals of our work included measurement of the excitation/emission properties of fluorescent pigments; collaboration in biochemical identification of the pigments, and development of in situ instruments and techniques for fluorescence imaging and measurement. Specific project results include: collection and distribution of several hundred measurements of fluorescence and reflectance of corals and other benthic organisms; development of a rule-based classifier that could be used with multispectral imagery collected by the Fluorescence Imaging Laser Line Scanner to perform automated classification of benthic surfaces; refinement of a mathematical model combining measured and modeled data to quantify the contribution of fluorescence to the spectral signature and apparent color of corals; and characterization of the green-fluorescent pigment in corals.

1. LONG-TERM GOAL

The long-term goal of this research is to gain an understanding of the nature and significance of fluorescence and reflectance characteristics of benthic marine organisms in general, and coral reef cnidarians in particular. We wish to determine 1) how biological processes act to produce the optical properties and 2) how optical measurements can be used to provide insight into biological state or process.

2. OBJECTIVES

Fieldwork for the CoBOP project was completed in FY00. The primary objective for FY01 was to analyze data to produce manuscripts for peer-review publication. This has focused on three topics:

- Development of a rule-based classifier that could be used with multispectral imagery collected by the Fluorescence Imaging Laser Line Scanner (FILLS) to perform automated classification of benthic surfaces;
- Refinement of a mathematical model combining measured and modeled data to quantify the contribution of fluorescence to the spectral signature and apparent color of corals;
- Characterization of the green-fluorescent pigment in corals.

In addition, data collected during the CoBOP field trips has been processed and organized so that it is accessible to collaborating researchers and to others outside the CoBOP program.

3. APPROACH

A set of classification rules for the FILLS imagery was developed using the ENVI software package (Research Systems Inc.). FILLS creates a multispectral image data set by scanning a blue laser beam across the sea floor and recording the light returning to four synchronized detectors. One of the detectors records the reflected laser light, while the other three record fluorescence in green, orange, and red wavelength bands. The rules were developed by examining the data values of the three fluorescence bands for recognizable bottom features in the image, writing routines that separated the data on the basis of those pixel values, and iteratively refining the rules based on observation of how well the classified image corresponded to the original image.

The modeling effort to determine the contribution of fluorescence to the spectral signature and apparent color of corals is related to a broader effort in this area. Initial results were presented as a poster at the 1999 conference of the American Society for Limnology and Oceanography (ASLO) (Mazel et al., 1999). A related effort evaluating the contribution of fluorescence of carbonate sediments and chlorophyll in benthic microalgae to upwelling light

was presented as a poster at the Ocean Optics XV conference (Mazel, 2000). The approach separates the reflectance and fluorescence components (Fux and Mazel, 1999; Fuchs, 2001) and computes their relative contributions to the light leaving the sample surface under various illumination conditions. Hydrolight 4.0 (Mobley, 1994) was used to model the illumination conditions. The model considers the significance of variations in depth, sun angle, and fluorescence efficiency. In addition to computing a composite (fluorescence + reflectance) spectral exitance curve, the resulting spectrum is processed using standard colorimetric techniques to compute coordinates on a standard chromaticity diagram. These in turn can be interpreted in terms of how the spectral distribution would be perceived by a human observer.

The work with the green fluorescence in corals is part of our ongoing investigation of fluorescent pigments. Some of the fluorescent pigments in Indo-Pacific corals have been identified (Matz et al., 1999) as forms of what is known as 'green-fluorescent protein'. This term is applied to a class of proteins, first discovered in a hydromedusa, in which the fluorescence is inherent to the protein and not associated with an attached chromophore. These proteins, called GFP for short, are of tremendous value in biochemical and molecular genetic studies. Our work, which has been a collaboration with the CoBOP groups headed by Michael Lesser (Univ. of New Hampshire) and Paul Falkowski (Rutgers Univ.) has demonstrated that several of the Caribbean coral fluorescent pigments we are studying are also forms of GFP. This work has been a combination of applied spectroscopy and biochemical isolation and characterization.

4. WORK COMPLETED

The bulk of the in situ and laboratory spectral data collected during the CoBOP project has been post-processed, checked for quality, and placed on an open ftp site. Three manuscripts have been prepared for submission to a special issue of the journal *Limnology and Oceanography* that will be dedicated to shallow-water optics.

5. RESULTS

The assignment of FILLIS multispectral fluorescence image pixels to bottom type categories based on the responses in the three fluorescence channels is illustrated in Figure 1. Once pixels were assigned to functional groups, bottom cover statistics were computed in several ways. In addition to using the full image, subsets of pixels were selected to simulate conventional diver survey techniques including line transects, grid samples, and photographic quadrats. The results of the imagery analysis compared well with bottom cover statistics derived from diver surveys.

The modeling approach to the contribution of fluorescence to the color of corals appears to be robust, and to produce results that correspond to in situ measurements of the spectrum of light leaving the surface of the sample, and to diver perceptions of the appearance of corals. (Figure 2).

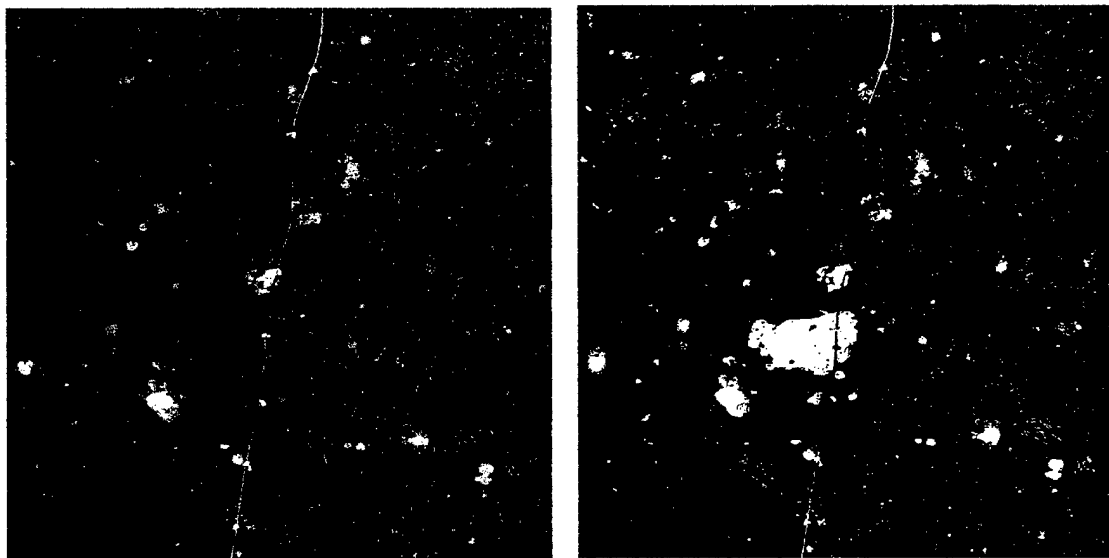


Figure 1. Original FILLs pseudocolor image (left) and the same image with pixels assigned to bottom types: green = sand; white = corals; bright and dull red = gorgonians; blue = red algae; purple = vase sponge; black = shadows; fish and sponge; pink = unclassified.

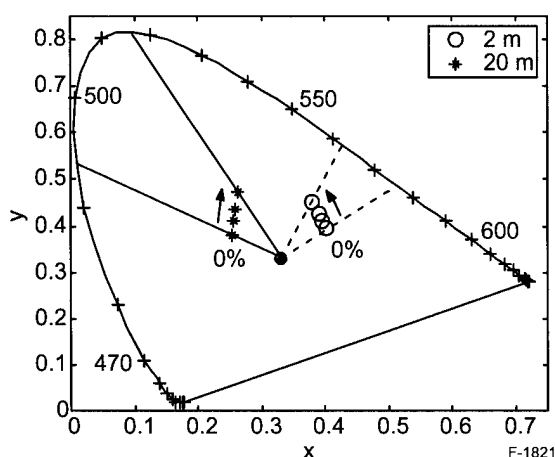


Figure 2. CIE chromaticity diagram illustrating the influence of green fluorescence pigment on the perceived color of a coral. Points are shown for four fluorescence efficiencies ranging from 0 to 10%, at depths of 2 m (open circles) and 20 m (asterisks). The movement of the points on the diagram is toward the green part of the spectrum, indicating that the effect of the increasing fluorescence is to make the corals appear more green, as expected. The effect is greater at the deeper depth.

Spectroscopic measurements and biochemical analysis proved that the pigment commonly found in Caribbean corals, designated '515' in Mazel (1997) for the approximate location of its spectral peak, is a variant of GFP. The pigment is widespread in Caribbean species. An important conclusion of the work is that spectroscopic measurements indicate that the GFP appears to have no impact on chlorophyll fluorescence. It has been suggested by other

researchers that the fluorescent pigments might act to shield the zooxanthellae in the coral tissues from high light levels (Salih et al., 2000), or that the fluorescence might act to provide extra photons for photosynthesis (Schlichter and Fricke, 1990). Our measurements of excitation spectra for chlorophyll fluorescence in many corals, both with and without high levels of GFP, show no evidence of either photon addition or removal by GFP.

6. IMPACT/APPLICATION

The measurement and analysis of benthic spectral properties will contribute to the understanding of the relation between biology and optics. These baseline spectra will also assist in the effort to use optical remote sensing to probe benthic biological systems and will be used in radiative transfer modeling.

7. TRANSITIONS

Spectral measurements of sediment and seagrass reflectance from Lee Stocking Island (LSI), Bahamas, have been supplied to collaborating researchers Curt Mobley (Sequoia Scientific), Ken Voss (Univ. of Miami, and Andy Farmer (Univ. of South Florida). Spectral measurements of coral reflectance have been supplied to Michael Lesser (Univ. of New Hampshire).

Outside the CoBOP group, the spectral reflectance data sets for all substrates at LSI were supplied to Jim Lange, Veridian/ERIM-International, for inclusion in a database being developed for the NAIC (National Air Intelligence Center) Spectral Exploitation Center (NSEC). This database will comprise a spectral library for use by the intelligence community in the analysis of hyperspectral imagery. The data set was also made available to Tony Filippi, a graduate student at the Center for GIS & Remote Sensing, Dept. of Geography, University of South Carolina. For his doctoral dissertation Tony is analyzing hyperspectral imagery collected at LSI by the Naval Research Laboratory's Portable Hyperspectral Imager for Low Light Spectroscopy (PHILLS).

8. RELATED PROJECTS

We are developing an improved version of the diver-operated spectral measurement instrument (DiveSpec) for more general use by CoBOP collaborators and other researchers (Mazel, 2001).

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